

**A COMPREHENSIVE REVIEW ON HIGH PERFORMANCE LIQUID
CHROMATOGRAPHY (HPLC)****Snehal Dattatray Domale*¹, Gauri Patil², Rajendra Patil³**¹Department of Pharmaceutical Quality Assurance, Delonix Society's Baramati College of Pharmacy, Barhanpur, Tal – Daund, Dist- Pune, Maharashtra, India.²Department of Pharmaceutical Quality Assurance, Delonix Society's Baramati College of Pharmacy, Barhanpur, Tal – Daund, Dist- Pune, Maharashtra, India.³Department of Pharmaceutical Chemistry, Delonix Society's Baramati College of Pharmacy, Barhanpur, Tal – Daund, Dist- Pune, Maharashtra, India.***Corresponding Author: Snehal Dattatray Domale**Department of Pharmaceutical Quality Assurance, Delonix Society's Baramati College of Pharmacy, Barhanpur, Tal - Daund, Dist- Pune, Maharashtra, India. DOI: <https://doi.org/10.5281/zenodo.21472134>**How to cite this Article:** Snehal Dattatray Domale*¹, Gauri Patil², Rajendra Patil³. (2026). A Comprehensive Review on High Performance Liquid Chromatography (Hplc). European Journal of Pharmaceutical and Medical Research, 13(7), 618–623.

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ABSTRACT

High-Performance Liquid Chromatography (HPLC) is one of the most extensively utilized analytical techniques used for the separation, identification, and quantification of compounds in complex mixtures. owing to its high sensitivity, accuracy, reproducibility, and versatility, HPLC has become an indispensable tool in pharmaceutical, biopharmaceutical, biomedical, environmental, food, and forensic analyses. The technique operates on the differential distribution of analytes between a stationary phase and a mobile phase under high pressure, resulting in efficient separation of components. Continuous advancements in column technology, detector systems, and data-processing software have significantly improved chromatographic performance and analytical throughout. Recent developments such as ultra-high-performance liquid chromatography (UHPLC), hyphenated techniques, and automated systems have further expanded the applications of HPLC in modern analytical laboratories. This review provides a comprehensive overview of the fundamental principles, instrumentation, operational mechanisms, and analytical applications of HPLC, highlighting its significance in contemporary scientific research and quality-control practices.

KEYWORDS: HPLC, Chromatographic Separation, Pharmaceutical Analysis, UHPLC, Quality Control.**1.1 INTRODUCTION**

Chromatography is one of the most important analytical techniques used for the separation, identification, and quantification of components present in complex mixtures. Since its introduction in the early twentieth century, chromatographic science has undergone remarkable advancements, leading to the development of highly efficient separation methods. Among these techniques, High-Performance Liquid Chromatography (HPLC) has emerged as one of the most powerful and widely adopted analytical tools due to its superior resolution, accuracy, sensitivity, and reproducibility.

HPLC evolved from conventional liquid chromatography to address the limitations associated with low separation

efficiency, long analysis times, and poor reproducibility. The introduction of high-pressure pumping systems, improved column packing materials, and advanced detection technologies significantly enhanced chromatographic performance, transforming HPLC into a cornerstone of modern analytical chemistry. Today, HPLC is routinely employed for the qualitative and quantitative analysis of a wide variety of compounds ranging from small organic molecules to complex biological macromolecules.

The fundamental principle of HPLC is based on the differential interaction of analytes between a stationary phase and a liquid mobile phase. As the sample passes through a chromatographic column under high pressure,

individual components are separated according to their physicochemical properties, including polarity, molecular size, charge, and affinity for the stationary phase. This mechanism enables efficient separation of closely related compounds with high precision and reliability.

Over the past few decades, HPLC has become indispensable in pharmaceutical research and development, where it is extensively used for drug discovery, formulation development, impurity profiling, stability testing, pharmacokinetic studies, and quality control. Beyond pharmaceutical applications, the technique plays a vital role in clinical diagnostics, biotechnology, environmental monitoring, food safety assessment, forensic investigations, and natural product analysis. Its versatility and adaptability have made it a preferred analytical method across both academic and industrial laboratories.

Continuous technological innovations have further expanded the capabilities of HPLC. Advancements in

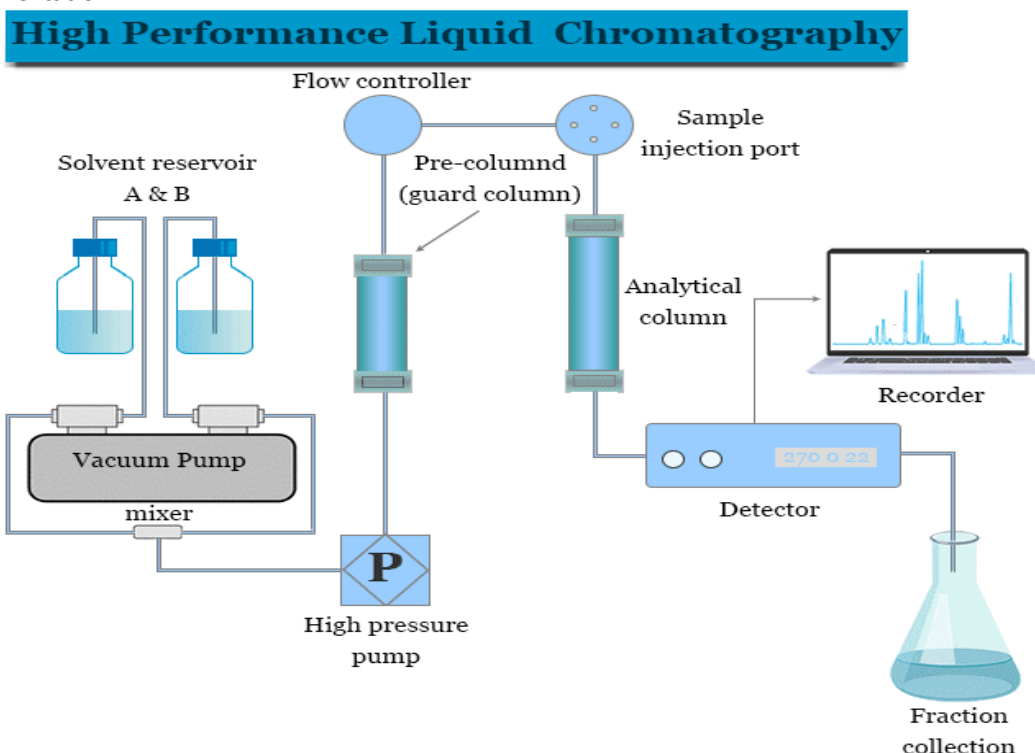
column chemistry, detector design, computerized data acquisition systems, and sample preparation techniques have improved analytical sensitivity, speed, and robustness.

In addition, the development of Ultra-High-Performance Liquid Chromatography (UHPLC) and hyphenated techniques such as HPLC–Mass Spectrometry (HPLC–MS) has enabled more comprehensive characterization of complex samples while reducing analysis time and solvent consumption.

Principle

High-Performance Liquid Chromatography (HPLC) is an analytical technique based on the differential distribution of analytes between a mobile phase and a stationary phase, resulting in separation due to variations in solute stationary phase interactions and solute mobile phase solubility.

1.2 Instrumentation



Mobile Phase

- The mobile phase is the liquid solvent (or mixture of solvents) that flows continuously through the chromatographic column, carrying the analytes along with it.
- It plays a critical role in determining the retention time, peak shape, and resolution of the analytes.

Characteristics of Mobile Phase

- Must be chemically inert and compatible with the stationary phase.

- Should have low viscosity to allow smooth flow under high pressure.
- Must be high purity (HPLC-grade) to avoid interference and noise.
- Should dissolve the sample effectively.

Types of Mobile Phase

- ✓ **Isocratic elution:** Composition of the mobile phase remains constant throughout the run.
- ✓ **Gradient elution:** Composition changes during the analysis to improve separation of complex mixtures.

Example

In reverse-phase HPLC (RP-HPLC), a commonly used mobile phase is a mixture of:

- Water (often buffered to control pH)
- Organic solvent such as methanol or acetonitrile

Types Of HPLC

1. Normal Phase Chromatography [NP]
2. Reverse Phase Chromatography [RP]

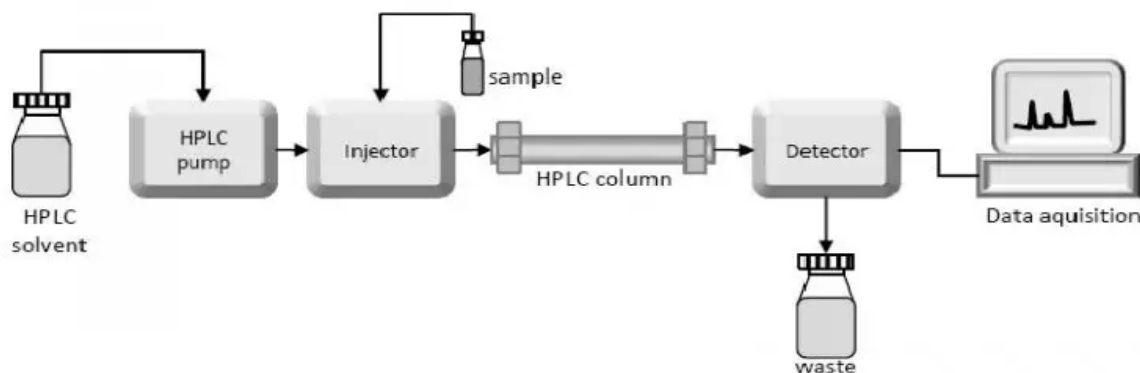
1. Normal Phase Chromatography [NP]

- Normal-phase chromatography is a type of liquid chromatography that uses a **polar stationary phase** (such as silica) and a **non-polar mobile phase** (such as hexane). Separation occurs because polar compounds interact more strongly with the stationary phase.
- Therefore, **non-polar compounds elute first**, followed by compounds with increasing polarity.
- **For example**, a non-polar compound like hexane will elute before a more polar compound like ethanol.

- This method is useful for separating compounds with low to medium polarity that dissolve in non-polar solvents.
- Highly polar or water-soluble compounds (such as glucose) are not suitable for this method.

2. Reverse Phase Chromatography [RP]

- Reversed-phase chromatography is the most commonly used type of liquid chromatography.
- It uses a **non-polar stationary phase** (such as C18) and a **polar mobile phase** (such as water mixed with methanol or Acetonitrile).
- Separation is based on the attraction between non-polar parts of compounds and the stationary phase.
- Thus, **polar compounds elute first**, and non-polar compounds elute later.
- **For example**, a polar compound like caffeine elutes before a non-polar compound like a lipid. This method is suitable for separating compounds with medium to high polarity

1.3 Components of High Performance Liquid Chromatography (HPLC)**1. Mobile Phase Reservoir**

- The mobile phase reservoir serves as the storage unit for the solvent used in the HPLC system
- It ensures a continuous, clean, and consistent supply of the mobile phase, often incorporating filtration to remove particulate impurities and maintain stable analytical conditions.

2. Pump

- The pump is responsible for delivering the mobile phase through the system at a constant flow rate and high pressure
- This consistent flow is essential for achieving reproducible separations and maintaining system stability.

3. Injector

- The injector introduces a precise and reproducible volume of the sample into the mobile phase stream.

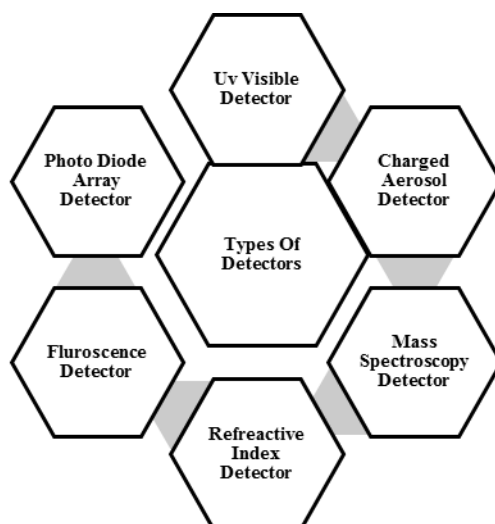
- Accurate injection is critical to ensure consistency and reliability of analytical results

4. Column

- The column is considered the core component of the HPLC system, as it is the site where separation of analytes occurs.
- It is packed with a stationary phase specifically selected based on the nature of the compounds being analysed.

5. Detector

- The detector monitors the compounds as they elute from the column and converts their presence into measurable signals.
- These signals are used for both identification and quantification of analytes.



Parameters	1.Uv Visible Detector	2.Charged Aerosol Detector	3.Mass Spectroscopy Detector	4.Fluroescence Detector	5.Refractive Index Detector
Principle	Measures The absorbance of UV/visible light at a selected wavelength.	Measures The absorbance across multiple wavelengths simultaneously using diode array.	Detects ions based on mass-to-charge ratio (m/z)	Measures emitted light after excitation of analyte	Measures change in refractive index of eluent vs sample
Selectivity	Moderate Only Uv Active Components	Higher than UV (spectral info aids identification)	Extremely high (molecular specificity)	Very high (only fluorescent compounds)	Low (almost all compounds detected)
Sensitivity	Moderate	Moderate	Very High	Very High	Low
Chromophores Requirement	Required	Required	Not Required	Not required	Not Required
Applications	Pharmaceuticals, organic compounds	Peak purity, Impurity profiling	Drug Analysis, Metabolomics	Trace analysis, biomolecules	Sugar Polymers Alcohol
Limitations	Cannot Detect nonUV Absorbing Compound	Provides full spectra, peak purity analysis	Very expensive, complex operation	Limited to fluorescent compounds	Low sensitivity, not suitable for gradients

1.4 Applications of High-Performance Liquid Chromatography (HPLC)

High-Performance Liquid Chromatography (HPLC) is one of the most widely utilized analytical techniques for the separation, identification, and quantification of chemical compounds in complex mixtures. Due to its high sensitivity, accuracy, and reproducibility, HPLC has found extensive applications across numerous scientific and industrial fields.

A. Biopharmaceutical Analysis

HPLC is an important analytical technique in the biopharmaceutical sector for evaluating complex biological products, including proteins, peptides, antibodies, and vaccines. It is used to assess product quality, detect contaminants and degradation compounds, and ensure stability during production and storage. The technique also supports process monitoring and quality assurance, helping maintain the safety, effectiveness, and uniformity of biopharmaceutical medicines.

B. Pharmaceutical Analysis

In the pharmaceutical sector, HPLC plays a critical role in drug development, formulation, and quality assurance. It is routinely employed for the determination of active pharmaceutical ingredients, impurity profiling, stability testing, and dissolution studies. The technique also supports pharmacokinetic and bioequivalence investigations by enabling precise measurement of drug concentrations in biological samples.

C. Clinical and Biomedical Research

HPLC is extensively used in clinical laboratories for therapeutic drug monitoring and biomarker analysis. It facilitates the detection and quantification of metabolites, hormones, vitamins, and other biologically important compounds in body fluids. Furthermore, the technique contributes significantly to metabolomic and proteomic studies aimed at understanding disease mechanisms and identifying diagnostic markers.

D. Environmental Monitoring

Environmental scientists rely on HPLC for the assessment of contaminants present in water, soil, and air. The method is particularly effective for detecting pesticides, herbicides, pharmaceutical residues, and other organic pollutants at trace levels. Its high selectivity makes it valuable for monitoring environmental quality and evaluating ecological risks.

E. Food and Beverage Analysis

The food industry utilizes HPLC to ensure product quality, safety, and authenticity. It is commonly applied for the determination of vitamins, amino acids, sugars, preservatives, artificial colorants, and contaminants such as mycotoxins and pesticide residues. Additionally, HPLC assists in detecting food adulteration and verifying product labeling claims.

F. Forensic Investigations

In forensic science, HPLC serves as a powerful analytical tool for the identification of drugs, toxins, and other chemical substances in biological and non-biological samples. The technique supports criminal investigations, toxicological examinations, and anti-doping programs by providing reliable qualitative and quantitative results.

G. Biotechnology and Life Sciences

HPLC is widely employed in biotechnology for the purification and characterization of proteins, peptides, nucleotides, and other biomolecules. It contributes to biopharmaceutical research, vaccine development, and the production of recombinant therapeutic products by ensuring product purity and consistency.

H. Agricultural Applications

Agricultural research benefits from HPLC through the analysis of pesticide residues, plant metabolites, and naturally occurring bioactive compounds. The technique aids in crop quality assessment, environmental impact studies, and the development of safer agricultural practices.

I. Industrial and Chemical Analysis

Various manufacturing industries use HPLC for process monitoring, raw material evaluation, and final product quality control. It is applied in the analysis of polymers, dyes, petrochemicals, and specialty chemicals, helping to maintain product standards and optimize production efficiency.

J. Emerging Applications

Recent advances in HPLC technology, including its integration with mass spectrometry and the development of ultra-high-performance liquid chromatography (UHPLC), have expanded its applications in genomics, proteomics, nanotechnology, and personalized medicine. These innovations continue to enhance analytical sensitivity, speed, and reliability, making HPLC an indispensable tool in modern scientific research.

1.5 Advantages of High Performance Liquid Chromatography (HPLC)**A. High Separation Efficiency**

HPLC provides excellent separation of compounds present in complex mixtures. Its high-resolution capability allows closely related substances to be distinguished and analysed accurately, making it valuable in pharmaceutical, biological, and environmental studies.

B. High Sensitivity

HPLC can detect and measure compounds present in very small quantities. This high sensitivity is particularly useful for trace-level analysis of drugs, impurities, metabolites, and environmental contaminants.

C. High Accuracy and Precision

The technique produces reliable and consistent results with minimal variation between analyses. This ensures accurate quantification of analytes and supports quality control and regulatory requirements.

D. Wide Applicability

HPLC can analyse a diverse range of compounds, including non-volatile, polar, ionic, and thermally unstable substances. This versatility makes it suitable for applications across multiple scientific disciplines.

1.6. Limitations of High-Performance Liquid Chromatography (HPLC)

Despite its widespread use and numerous advantages, High-Performance Liquid Chromatography (HPLC) has several limitations that should be considered during analytical method selection and application.

A. High operational Cost

The acquisition and maintenance of HPLC systems require substantial financial investment. In addition to the cost of the instrument, expenses associated with columns, detectors, maintenance, and high-purity solvents contribute significantly to overall operating costs.

B. Extensive sample preparation

Many analytical samples require preliminary treatment before chromatographic analysis. Procedures such as extraction, filtration, dilution, and purification can be labour intensive, time-consuming, and may introduce variability or analytical errors if not performed properly.

C. Large solvent requirements

HPLC methods typically consume considerable volumes of organic solvents. This not only increases operational expenses but also generates chemical waste that requires appropriate disposal, raising environmental and safety concerns.

D. Challenges with high complex samples

Although HPLC provides excellent separation efficiency, samples containing a large number of closely related compounds may not be completely resolved in a single

chromatographic run. In such cases, advanced techniques such as multidimensional chromatography or HPLC coupled with mass spectrometry may be necessary.

E. Requirement of skill personnel

Successful operation of HPLC instruments, method optimization, data interpretation, and troubleshooting demand specialized training and technical expertise. Insufficient experience can negatively affect analytical performance and result reliability.

F. Column Deterioration

Chromatographic columns gradually lose efficiency due to contamination, chemical degradation, or mechanical wear. Regular maintenance and periodic replacement of columns are therefore necessary to maintain analytical accuracy and reproducibility.

G. Detector Limitation

Many HPLC detectors are selective and may only respond to specific classes of compounds. While universal detectors are available, they often exhibit lower sensitivity compared to compound-specific detectors, potentially limiting the detection of trace-level analytes.

CONCLUSION

High-Performance Liquid Chromatography (HPLC) is one of the most important analytical techniques used for the separation, identification, and quantification of compounds in complex mixtures. Its high sensitivity, accuracy, precision, and reproducibility make it an essential tool in pharmaceutical, biopharmaceutical, clinical, environmental, food, and forensic analysis. Continuous advancements such as UHPLC, hyphenated techniques, and improved detector technologies have further enhanced its analytical performance and expanded its applications. Although HPLC has limitations, including high operational costs and the need for skilled personnel, its advantages outweigh these challenges. Therefore, HPLC continues to play a vital role in research, quality control, and regulatory compliance and is expected to remain a cornerstone of modern analytical science.

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